**SYNOPSIS**

**Ph.D in Zoology**

# An *in vitro* study on the decolourisation of Corafix YellowGD3R dye using green seaweed *Caulerpa taxifolia* and its toxicological evaluations

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## INTRODUCTION

Water as the elixir of life, emphasizes its fundamental role in sustaining not only human life but also the lives of millions of other species on our planet. The textile industry plays a major role in causation of pollution due to the release of untreated dyeing effluents into aquatic and terrestrial ecosystems (Bhatia *et al.,* 2018). Dyes represent an important group of organic pollutants because they are utilized in several areas such as rubber, textiles, food, pharmacy, leather, paper and cosmetics industries (Kamranifar *et al.,* 2018). Amidst all, azo dye occupies the largest class comprising nearly 60 – 70% of all the dyeing materials produced annually. Reactive dyes are composed of azo-based chromophores which are widely used in various industrial applications because of their bright colors, excellent colorfastness, ease of application, solubility, low cost, minimal energy consumption, good sensitivity to sunlight and water (Benkhaya *et al.,* 2022). Reactive dyes are unique among dye classes because they form covalent bonds with textile fibers, such as cotton. This covalent bonding makes them highly resistant to conventional wastewater treatment processes, which rely on physical and chemical methods to remove pollutants. Due to their complex structure and xenobiotic nature, they persist in the aquatic system and thereby difficult to remove or degrade (Cao *et al.,* 2017). Corafix Yellow GD3R (CYGD3R) is stable, water soluble, anionic azo reactive dye that is widely used in the textile, pharmaceutical, printing, paper, and food sectors, as well as in research. Inhalation of Corafix Yellow GD3R causes allergy, hypersensitivity, dermatitis and cancer. As a result, removing Corafix Yellow GD3R from wastewater in an effective and economic mode is vital before discharged into the natural environment. Physico-chemical processes such as coagulation, flocculation, electrochemical methodology, oxidation, filtration, solvent extraction, reverse osmosis, membrane separation, ion-exchange, precipitation and photo degradation have been used to treat textile industrial wastewater due to their faster treatment rate, but they are proved to be expensive, not eco-friendly and generates large amount of sludge. To combat this, research and development in this field have focused on exploring biological such as bacteria, fungi, yeast, algae, enzymes (Mohanty and Arvind Kumar *et al*., 2021), plants and seaweeds (Arumugam *et al.,* 2018) for the removal of colour from the textile dyeing effluent. Among the biologicals, usage of seaweed in the textile dye remediation is potentially advantageous due to its ease, cost-effectiveness and eco- friendly nature. Seaweeds can easily grow in both fresh and saline water depending on the climate, requires less financial input to cultivate and generate least amount of sludge. Seaweeds are promising sustainable biosorbents due to their natural abundance, surface properties, high ability for biosorption of pollutants and low-cost. In the present

study, the green macroalgae *Halimeda gracilis, Halimeda macroloba* and *Caulerpa sp.* has been selected to assess their decolourisation efficacy against CYGD3R. Furthermore, in order to evaluate the efficacy of wastewater treatment systems and better assure the safety of the receiving media, monitoring of chemical parameters and effluent toxicity prior to release into the environment is essential.

Thus, with this background information, the present study was aimed to explore the potential of *Caulerpa taxifolia* as a biosorbent for the removal of CYGD3R from aqueous solutions under optimized conditions and to assess its applicability on biological models. The objectives of the present study are follows.

- To determine the efficacy of the selected seaweeds to decolourise CYGD3R.
- To optimize the process parameters to achieve maximum decolourisation using Response Surface Methodology (RSM)
- To assess the adsorption mechanism and adsorbate- adsorbent interactions.
- To analyse the effectiveness of the dye adsorbed seaweed in terms of desorption and regeneration.
- To assess the physicochemical characterization and the toxicity of untreated and treated dye solutions using biological models.
- To analyse the efficacy of the seaweed on real textile industrial effluent.

## MATERIALS AND METHODS

### Preparation of adsorbate and biosorbent

The azo reactive dye, CYGD3R (CAS NO: 171599-84-1, molecular weight: 1160.5071g/mol, molecular formula: $C_{34}$-$H_{29}$-CI-$N_{12}$-$O_{19}$-$S_6$.x-Na) was obtained from the local dyeing industry, Perundurai, Tamil Nadu, India. The stock solution was made by dissolving 1g of CYGD3R dye in 1L of water and left overnight to completely dissolve the dye powder and further dilutions were made.

The seaweeds namely *Halimeda gracilis, Halimeda macroloba* and *Caulerpa* sp. were procured from R. K. Algae Research Project Centre, Mandapam, Ramanathapuram, Tamil Nadu, India. They were transferred to the labotory in polythene bag filled with saline water and washed thoroughly with running tap water to remove the sand, impurities and larvae adhered on it. Further the seaweeds were washed with distilled water to ensure from the rid of contaminants on their surface. The washed seaweeds were shade dried at room temperature for 10 days and the completely dried seaweeds were pulverized into fine powder and stored in air tight container for further studies (Klijinstra *et al.,* 2020).

### Screening of dyes for decolourisation using seaweeds

A pilot study was performed to screen the efficacy of the seaweeds against CYGD3R dye using batch adsorption method (Abar *et al*., 2009). The dye was taken at varying concentrations (100 - 700mg/L), inoculated with 100 - 800mg/L of each biosorbent separately and the pH was varied (2 - 12) and allowed to incubate for five days at room temperature. At the end of the incubation time, the percent dye removal was recorded at 530nm spectrophotometrically (Hitachi U 2800, Tokyo, Japan). The decolourisation percentage was evaluated using the following equation,

$$\text{Decolourisation (\%)} = \frac{(A_i) - (A_t)}{(A_t)} \times 100$$

where, $A_i$ was the initial absorbance and $A_t$ is the absorbance at incubation time t. The seaweed which exhibited maximum decolourisation percentage was selected for further studies and subjected to identification.

### Identification of the selected seaweed

The seaweed which removed maximum colour was submitted to Botanical Survey of India, Tamil Nadu Agricultural University, Coimbatore, Tamil Nadu, India, for further confirmation and authentication.

### Characterization of the selected seaweed

To determine the surface charge of the biosorbent, batch equilibrium approach was performed to

calculate the point of zero charge ($pH_{pzc}$) using NaCl. The specific surface area, total pore volume and pore size distribution of the biosorbent was analysed using BET and BJH method (Micromeritics Instrument Corporation, Model-3Flex, Norcross, GA, USA) (Li *et al*., 2019).

**Optimization of process parameters for CYGD3R decolourisation using Response Surface Methodology (RSM)**

Response surface methodology (RSM) combined with based Box-Behnken design (BBD) was established using Design Expert software (STAT EASE 360). In order to evaluate the effect of operating parameters on the decolourisation efficiency of CYGD3R, four independent variables namely dye concentration (A), biosorbent concentration (B), pH (C) and incubation time (D) were selected for the study. The batch adsorption experiment was performed at room temperature by varying the dye (50-300 mg/l) and biosorbent (200-700 mg/l) concentrations at different incubation time (24 -144 hours) and the pH (4 - 10) was adjusted using 0.1N HCl and 0.1N NaOH. Each independent variable was evaluated at lower (-) and higher (+) levels.

Regression analysis was conducted using second order polynomial equation to reveal the interaction of the input variables with one another in the decolourisation of CYGD3R. Analysis of Variance (ANOVA) was carried out to determine the applicability and relevance of the predicted model. The significance of the entire design model was assessed using the F value (Fisher variation ratio), lack of fit and probability value ($p < 0.0001$). The coefficient of determination ($R^2$) was used to determine the correlation between the experimental and predicted values. The response surface and contour plots of the model reveals the interaction between the experimental variables and responses, and thereby the optimal values of the process variables were attainted (Venkataraghavan *et al.,* 2020).

In order to maximize the responses, the desirability function was used to determine the optimal predicted conditions for CYGD3R decolourisation. The experiment was run in triplicates and the predicted values were compared in order to confirm the decolourisation of CYGD3R under ideal conditions.

**Adsorption isotherms, kinetics and thermodynamic studies for CYGD3R removal**

The experimental data from the batch adsorption investigation was applied to Langmuir and Freundlich isotherm models to determine the monolayer and multilayer adsorption of CYGD3R onto the selected seaweed. Similarly, the data was fitted to pseudo first-order, pseudo- second-order and intraparticle diffusion models to identify the mechanism of the adsorption process. Under optimized conditions, thermodynamic parameters such as Gibbs free energy (ΔG°), enthalpy (ΔH°) and entropy (ΔS°) were studied to validate the nature of adsorption process (Mokhtara *et al*., 2017).

**Analyses of adsorbate- biosorbent interactions**

To assess the changes in adsorption spectra, the untreated and treated dye solutions were subjected to UV–Visible spectrophotometer (Shimadzu / 206 – 26300 - 48, Japan) at 300–800 nm. Fourier Transform Infrared Spectroscopy (Shimadzu (Miracle 10), Japan) was performed at 4000 – 400 $cm^{-1}$ to determine the shifts in the functional groups involved during the process of dye decolourisation. The surface morphology of the biosorbent before and after dye decolourisation was analysed using Scanning Electron Microscopy and Energy Dispersive X-ray (TESCAN — MIRA3 XMU, Czech Republic). The X-ray diffraction was used to determine the nature of dye unloaded and loaded biosorbent scanned at the ratio of 3°/min over the range of 20°- 80° (2θ) (Philips PW-3710, England) (Vigneshpriya *et al*., 2019).

**Desorption and regeneration studies**

To determine the desorption efficiency, the dye-loaded biosorbent (500 mg/l) was added into different eluting agents namely 0.1M NaOH, 0.1M $HNO_3$, 0.1M HCl, and 0.1M $CH_3COOH$ separately. The contents were agitated at 200rpm for 3 days at room temperature (25°C) and the experiment was performed in three consecutive cycles. The desorption efficiency was calculated by determining the ratio between the amount of dye desorbed and the amount of dye adsorbed onto the biosorbent. After desorption, the dye-loaded biosorbent was thoroughly washed with distilled water thrice to remove any remaining traces of the eluting agents. The regenerated biosorbent is then mixed with the dye solution for the next adsorption cycle. After the completion of each adsorption cycle, the percent decolourisation of dye was calculated (Indah *et al.,* 2017).

**Physico-chemical analyses of the untreated and treated dye solutions**

The untreated or treated dye solutions were subjected to physico-chemical analyses (APHA, 2012). The physical (Colour, pH, Temperature, Electrical Conductivity, Total Solids, Total Dissolved Solids, Total Suspended Solids) and the chemical (Total Hardness, Total Alkalinity, Chemical Oxygen Demand, Biochemical Oxygen Demand, Sulphate, Phosphate, Nitrate, Fluoride, Iron and Chloride) parameters were analysed in the untreated and treated dye solutions and compared with the standard limits prescribed by BIS (Bureau of Indian Standards)(Bakar *et al.,* 2020).

**Toxicity assessments of untreated and treated dye solutions**

**Phytotoxicity**

To evaluate the toxic nature of the treated dye solution, phytotoxicity study was carried out using *Vigna radiata* as candidate plant. The seeds of green gram were initially surface sterilized with 0.1% sodium hypochlorite for 2 minutes and further washed thrice with distilled water and 10 seeds were

placed in the pot containing red soil and sand (Yadav *et al.,*2022). The seeds were periodically watered with tap water (Control $T_1$), untreated ($T_2$) and treated ($T_3$) dye solutions separately for 7 days. At the end of 7$^{th}$ day, the parameters such as germination percentage, shoot and root length, vigour index, germination rate index, germination speed and germination energy, tolerance index, percentage of phytotoxicity and phytotoxicity index were calculated.

**Cytotoxicity**

*Allium cepa* (2n=16) was selected as test indicator to carry out cytotoxicity analysis. Healthy onion bulbs were procured from the local market in Coimbatore, Tamil Nadu, India. The onion bulbs were divided into three groups, with five in each group. The onion bulbs were immersed in glass beakers containing 200ml of only tap water ($T_1$) as control, untreated ($T_2$) and treated ($T_3$) dye solutions separately for 5 days at room temperature. The test solutions were renewed for every 24h to ensure proper growth. After 5 days of treatment, the apical part of the root from onion bulb was cut, washed and immediately fixed in Carnoy's fluid (acetic acid and ethanol in 1:3 ratio) for 5min to study the nuclear aberration assay. The root tips were withdrawn from the fixing solution, washed twice with distilled water and further hydrolyzed with 1N HCl at 60ºC for 5min to soften the tissues. Further the root tips were stained with aceto-orecin for 15 min and the darkly stained apical root tips were subjected for slide preparation. The slides were examined for chromosomal aberrations in all the groups under light microscope and the images were captured (Domingues *et al.,*2020).

**Brine shrimp lethality assay**

The eggs of Brine shrimp (*Artemia salina*) were incubated overnight for hatching in a trough containing saline water with ambient light under laboratory conditions. After 24 h newly hatched larva numbering 10 was selected and added into saline water (90 ml) amended with 10 ml of untreated and treated dye solutions separately. For every 3 h time interval the mortality of *A. salina* was recorded upto 48 h. The evaluation was done by counting the number of dead larva and the percentage mortality was calculated using the formula given below (Eshghi and Kashi, 2022)

$$\text{Mortality (\%)} = \frac{\text{Number of dead brine shrimp}}{\text{Number of brine shrimp introduced}} \times 100$$

**Application of the seaweed on real industrial effluent**

The real textile industrial effluent was collected from the local dyeing industry, Perundurai, Tamil Nadu, India. Under optimized conditions the biosorbent was added into the effluent and the decolourisation percentage was determined (Naggar *et al.,* 2020).

## RESULTS AND DISCUSSION

### Preliminary screening, identification and characterization of dye decolourising seaweed

### Preliminary screening of dyes for decolourisation using seaweeds

A pilot study was conducted using the seaweeds namely *Caulerpa* sp.*, Halimeda gracilis* and *Halimeda macroloba* against the azo reactive dye, Corafix Yellow GD3R by varying the concentrations of dye (100-700mg/l) and biosorbent (100-800mg/l), pH (2-12), incubation time (1-5days) and 25ºC to 45ºC temperature. The results of pilot study revealed that *Caulerpa* sp. exhibited maximum dye decolourisation (97%) when compared with *H. gracilis* (88%) and *H.macroloba* (84%). Hence further decolourisation study was conducted using *Caulerpa* sp. against the dye CYGD3R.

### Identification of the selected seaweed

Based on the morphological features the seaweed was authenticated as *Caulerpa taxifolia* (voucher specimen no: BSI/SRC/5/23/2021/TECH/370) which belongs to the division Chlorophyta and the family *Caulerpaceae*.

### Characterization of the selected seaweed

The point of zero charge data denotes the pH level at which the biosorbent net charge is zero. The pHzpc of *C.taxifolia* was empirically determined at 5.4, which represent that the pH below 7 (acidic) is advantageous for anionic dyes. According to the BET and BJH analysis, the features of *C. taxifolia* were deduced from their adsorption-desorption isotherms. The plot reveals type IV isotherm, indicating that *C. taxifolia* is mesoporous (2 – 50nm) as per IUPAC classification. The results revealed that the specific surface area, total pore volume and pore size were found to be 9.92 $m^2 g^{-1}$,0.00198 $cm^3/g$ and 1.22 nm respectively.

### Optimization of process parameters for CYGD3R decolourisation using Response Surface Methodology (RSM)

Optimization was conducted using Box-Behnken Design (BBD), involving a total of 29 experimental runs based on the observed data. Based on the ANOVA results, the high statistical significance and usefulness of the developed model equation for CYGD3R biosorption was confirmed by a low probability value ($p<0.0001$) as well as high adequate precision value. The goodness of fit of the model was confirmed by coefficient of determination ($R^2$) which should be close to 1 for a good statistical model. In the present study the $R^2$ value was found to be 0.9908

which implies that more than 90% of the experimental data was compatible with data predicted by the model. A low CV value indicate that the deviation between experimental and predicted values are low. The larger F-value and the smaller P- value indicates a greater significance of the corresponding coefficients. The results of adequacy of the model reveals that the residuals were scattered around the straight diagonal line, close to the diagonal line, and the residuals were between the two red dashed lines, thereby demonstrating the accuracy of the model. In this study, the value of lambda (λ), which was frequently used to assume any transformation for normalization of data to boost the model relevance, was evaluated using a Box-Cox plot. The optimal value (λ) =1 associated with model design value suggested no transformation of the model, proving that the data is normally distributed and predicted. The Perturbation plot identifies the most influential factors affecting the response. The curve exhibited a smooth and symmetrical behaviour around the central level of each variable indicating a good impact in dye decolourisation. Three dimensional curves and their associated contour plots were examined in order to analyse the interaction between the variables through response surface analysis for CYGD3R decolourisation by *C. taxifolia*. The contour plot of the study reveals elliptical interaction between all the variables and thereby suggest the model is significant in the decolourisation process. To validate the results obtained from the statistical analysis of BBD, the experiment was carried out under optimal conditions (dye concentration - 100mg/L, biosorbent concentration - 500mg/L, pH - 8, incubation period - 72hrs) at room temperature obtained through RSM. The experimental results revealed that maximum decolourisation percentage of 97.43% which was quite close to the predicted value (96.87%) using RSM.

**Adsorption isotherms, kinetics and thermodynamic studies for CYGD3R removal**

The equilibrium data results were better aligned with the Langmuir isotherm compared to the Freundlich isotherm model, indicating a monolayer adsorption of CYGD3R on the surface of the biosorbent. The experimental data well fits with the pseudo-second-order kinetic model. The results of correlation coefficient ($R^2$) also inferred that the adsorption behavior of CYGD3R dye onto *C. taxifolia* follows pseudo second order kinetic model which is regulated by chemisorption involving valence forces through sharing or exchange of electrons between the dye and the biosorbent. Thus, kinetic study provides valuable insights into the process of adsorption, which can be useful for designing and optimizing dye removal from wastewater or other environmental applications. Since the pseudo first order and pseudo second order kinetic models cannot recognize the diffusion mechanism, the kinetic data were further evaluated to identify the steps involved in the adsorption

process through Weber Morris intraparticle diffusion. Deviation from the origin in the graph noted the linear line does not pass through the origin. Thereby depicting the intraparticle diffusion is not the only rate-limiting step in the adsorption process.

In thermodynamic parameter the negative value for Gibbs free energy (ΔG) and the positive values of enthalpy (ΔH) and entropy (ΔS) suggests that the adsorption is feasible, spontaneous, and endothermic in nature governed by chemisorption processes.

**Analyses of adsorbate- biosorbent interactions**

The results of the UV-Vis spectrum revealed the disappearance of the UV peaks in the dye treated solution, which exhibits the removal of CYGD3R by *C. taxifolia*. The FT-IR spectrum of dye unloaded seaweed revealed the presence of various functional groups such as hydroxyl, carboxyl, alkyl and alkane and their peak shifts in the FT-IR spectrum of dye loaded seaweed which showcases the involvement of the functional groups in the interaction with CYGD3R. The XRD spectrum of dye unloaded seaweed which exhibited clear crystalline peaks and the dye loaded seaweed exhibited low intensity peaks which might be attributed to the adsorption of CYGD3R by *C. taxifolia.*

The surface of the seaweed in SEM revealed rough which gets entrapped after exposure to the dye molecules. EDX spectrum of the dye unloaded seaweed was observed to have various elements namely carbon, oxygen, chlorine, sodium, potassium, silicon, sulphur and calcium whereas the dye loaded seaweed revealed the absence of sodium, potassium and chlorine which indicates the interaction of dye with the biosorbent.

**Desorption and regeneration studies**

The results for desorption studies revealed that recovery of the biosorbent was high (83%) in the first cycle of desorption using 0.1M nitric acid as an eluent and reduced in consecutive cycles and also in other eluents. The dye eluted seaweed was found to have high decolourisation (71%) percentage during the first cycle which was reduced in further cycles.

**Physicochemical analyses of untreated and treated dye solutions**

The levels of physicochemical parameters in untreated dye solution does not fall within the tolerance limits prescribed by BIS for the discharge into inland surface whereas the levels in treated dye solution falls within the prescribed limits and certain parameters shows slight deviations from the limits prescribed by BIS suggesting the potential of *C. taxifolia* in the treatment of treated dye solution.

**Toxicity assessments of untreated and treated dye solutions**

To evaluate the toxicity of untreated and treated dye solutions toxicity assessments such as phytotoxicity, cytogenotoxicity and brine shrimp lethality assay were performed.

**Phytotoxicity**

Results of the phytotoxicity study revealed that the treated dye solution exhibited a noticeable increase in germination percentage, shoot and root length, vigour index, germination rate index, germination speed and energy, whereas it was decreased in untreated dye solution. The low tolerance index, phytotoxicity percentage and phytotoxicity index in the treated dye solution indicates the non-toxic potential towards the seedlings of *Vigna radiata* as compared with control.

**Cytotoxicity**

The cytogenotoxicity were carried out using *Allium cepa* to identify the effects of untreated and treated dye solution through chromosomal aberrations and compared with control (tap water). The results revealed that onion root tips exposed to untreated dye solutions exhibited various chromosomal aberrations namely binucleate cells, sticky metaphase, laggard chromosomes, disturbed metaphase and anaphase which showcases the toxicity of untreated dye solution. The onion root tips obtained from treatment of control (tap water) and treated dye solution displayed normal stages of mitosis (prophase, metaphase, anaphase and telophase).

**Brine shrimp lethality assay**

Results of the brine shrimp lethality study revealed that as time increases the percent mortality increased in all the treatments i.e., within 48 h, 100%, 80% and 40% mortality was recorded when *A. salina* was exposed to untreated dye solution, treated dye solution and saline water (control) respectively.

**Application of the seaweed on real industrial effluent**

The textile dyeing effluent inoculated with *C. taxifolia* species recorded 82% colour removal at optimal conditions (biosorbent concentration - 500mg/L, pH - 8, incubation time - 72 hours and temperature - 25°C).

**Conclusion**

The study indicated that green seaweed *Caulerpa taxifolia* showed a great potential for decolourisation of Corafix Yellow GD3R from aqueous solutions. In batch studies, maximum dye

removal by *C.taxifolia* was observed at 100mg/L dye concentration, 500mg/L biosorbent concentration, pH 8 at 25ºC on 72 h of incubation period. Desorption studies proved that the recovery of dye was highly possible using 0.1M $HNO_3$ as eluent and recycling of treated dye solution for dyeing also controls the pollution level. The experimental data fitted well in the Langmuir isotherm, thus proving homogenous monolayer adsorption of CYGD3R on to the biosorbent. Analysis of data proves the chemisorption process by obeying pseudo-second- order kinetics and thermodynamic treatment of equilibrium data shows that the process of dye adsorption is feasible, spontaneous, and endothermic in nature. UV- Visible spectroscopy specified the biosorption of dye by the reduction/disappearance of absorption peaks. FT-IR analysis established the role of different functional groups for the sorption of the dye. The surface of the seaweed in SEM revealed rough which gets entrapped after exposure to the dye molecules. EDX analysis revealed the elements employed in the adsorption process. Toxicity investigations using *Vigna radiata*, *Allium cepa* and *Artemia salina* demonstrated that the treated dye solution is non-toxic in nature. The textile dyeing effluent under optimized conditions removed 82% of colour indicating the efficacy of *C. taxifolia* in practical applications. Thus the results of the present study reveals that exploration of *Caulerpa taxifolia* could be an eco-friendly, cost effective and an alternative approach for the removal of dyes from aqueous solutions.

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